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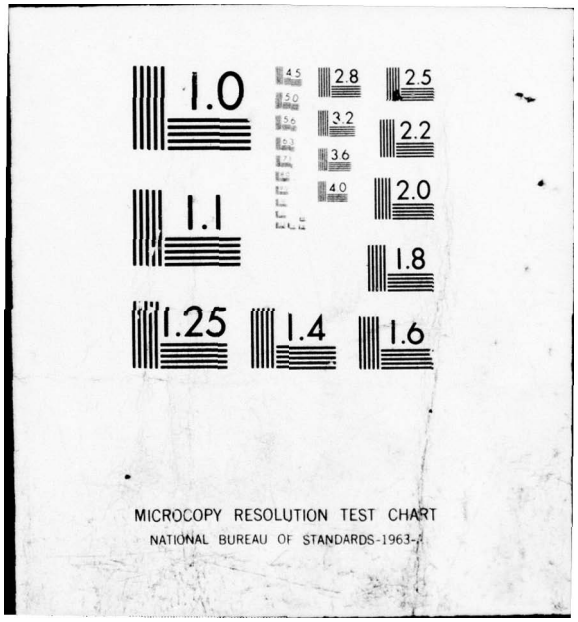
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REPORT NO. NADC-77308-60

RECOVERY OF MONKEYS FROM CARDIOGENIC SHOCK AFTER MYOCARDIAL INFARCTION WITH VENTRICULAR FIBRILLATION

Effects of PGB_x

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12 DECEMBER 1977

PHASE REPORT
CONTRACT NO. N00014-77-WR701-06

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Prepared for
OFFICE OF NAVAL RESEARCH
Biochemistry Program
Arlington, Virginia 22217

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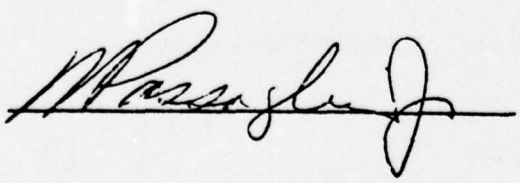
APPROVED BY:  DATE: 12/12/77

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REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: NADC-77308-60	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): RECOVERY OF MONKEYS FROM CARDIOGENIC SHOCK AFTER MYOCARDIAL INFARCTION WITH VENTRICULAR FIBRILLATION - Effects of PGB$_x$.	5. TYPE OF REPORT & PERIOD COVERED: Phase Report
6. PERFORMING ORG. REPORT NUMBER	
7. AUTHOR(s): E.T. Angelakos, R.L. Riley (Hahnemann Medical College, Philadelphia), B. David Polis	8. CONTRACT OR GRANT NUMBER(s): Contract No. N00014-77-WR701-06
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Aircraft & Crew Systems Technology Directorate, Naval Air Development Center, Warminster, PA 18974	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 12 35 p.
11. CONTROLLING OFFICE NAME AND ADDRESS: Office of Naval Research, Biochemistry Program, Arlington, VA 22217	12. REPORT DATE: 12 Dec 77 13. NUMBER OF PAGES: 31
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report): UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Cardiogenic Shock
Myocardial Infarction
Ventricular Fibrillation

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A polymeric, stable free radical derivative of 15-keto-prostaglandin B, named PGB$_x$, which conserves oxidative phosphorylation in mitochondria under degenerative conditions in-vitro, was studied for its effect on the survival of primates subjected to cardiogenic shock following ~~coronary ligation and~~ induced ventricular fibrillation (VF) in male rhesus monkeys (5-9kg) anesthetized with pentobarbital. VF was induced in all animals 20 minutes ~~after ligation~~ and maintained sequentially for 4, 6, 8, 12 and 24

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20. minutes in successive periods with 20-30 minute recovery intervals when the animal survived the fibrillation episode. Intra-cardiac norepinephrine (NE), cardiac massage (CM) and electrical defibrillation (EDF) were used to restore cardiac function in the controls. The experimental animals were given 1mg/kg PGB_X in addition. Recovery was established by the maintenance of effective blood pressure without exogenous support. In the control series the cumulative survival for the sequential fibrillation periods of 4, 6, 8, and 12 minutes was 60, 44, 31 and 25 percent. For the PGB_X treated series survival for equivalent VF periods was 100, 94, 94 and 88 percent. The difference was highly significant statistically. Evidence is presented also for a synergistic action between norepinephrine and PGB_X in the recovery from cardiogenic shock. Electron microscopy of isolated mitochondria in-vitro and of heart muscle sections in-vivo show a preservation of mitochondrial structural integrity by PGB_X under conditions which degenerate the controls. The data indicate that PGB_X significantly improved cardiac recovery after circulatory arrest due to VF in the presence of acute myocardial infarction.

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ACKNOWLEDGEMENTS

The authors are indebted to and acknowledge with appreciation the work of Mr. Ronald West in the surgery and experimental measurement with the monkeys; the statistical handling of the data by Professor John De Cani; and the sample preparation and electron microscopic interpretations by Dr. John Stasny.

INTRODUCTION

Intractable shock in acute myocardial infarction is a well recognized clinical problem, currently with no adequate effective therapy and a prognosis of imminent death. Although shock has been defined in mechanical terms of inadequate tissue perfusion, there is increasing evidence referring shock to conditions of biochemical abnormalities with insufficient energy for cellular survival because of decreased ATP production, mitochondrial damage, changes in membrane permeability, and lysosomal membrane rupture leading to irreversible cell damage and finally death (1). Myocardial cell death is believed to occur when intracellular ATP drops below 2.0μ moles/gram and the anaerobic metabolism of the ischemic heart cell stops (2). In studies on the ischemic - anoxia produced by the acceleration of rats at 20G, survival was markedly enhanced by drastic changes in pituitary - adrenal hormones which correlated with the maintenance of high levels of ATP in the brain (3,4). In subsequent studies on degenerative biochemical changes in anoxic stress (5,6) a new bioregulatory factor was discovered which has the unique property of conserving the mechanisms of oxidative phosphorylation of isolated mitochondria in-vitro under degenerative conditions leading to a complete loss of the oxidative energy transformation process (7). This factor, currently termed PGB_X, is a tetrameric condensation of prostaglandin B to form a new stable free radical molecule which has lost all the described properties of the parent prostaglandins.

When mitochondria are slowly degenerated by ageing at 0°C, followed by a brief additional Mg^{++} catalyzed degeneration at room temperature, a complete loss of oxidative phosphorylation activity occurs. The addition of PGB_x to the reaction preserves and restores oxidative phosphorylation to normal levels (7).

In studies with inhibitors or Ca^{++} competing for phosphorylation sites on the mitochondria, PGB_x acted to sustain oxidative phosphorylation. In the interplay between Ca^{++} and PGB_x an in-vitro control of the phosphorylation level could be achieved. All the effects of PGB_x were observed with so called "damaged" mitochondria. No effect of PGB_x was observed with normal, intact mitochondria. These findings suggested the use of PGB_x in-vivo for the amelioration and survival of cellular catastrophes involving mitochondrial damage resulting in shock and death which occur in ischemic - anoxia pathology.

The intent of the experimental design to be described was to evaluate the effect of PGB_x in the restoration of tissue and organ function after lethal periods of ischemia and hypoxia had rendered the organ intractable to the most effective therapeutic procedures prevalent. Based on preliminary studies, the experimental procedure involved evaluation of overall cardiovascular recovery and survival after a period of ventricular fibrillation in heart with a left ventricular infarction from a coronary ligation. This provided an insult of such magnitude that recovery in untreated animals is at best difficult and is associated with a high incidence of

mortality (8,9).

METHODS

Two species of monkeys, Rhesus (Macaca mulata) and African Green (Cercopithecus aethiops), were used in these studies.

The monkeys were anesthetized with pentobarbital (30mg/Kg) and a thorocotomy on the left side between the 4th and 5th intercostal space was performed under positive pressure artificial respiration. Catheters were placed in the thoracic or abdominal aorta for direct recording of blood pressure and heart rate, and in the vena cava for venoclysis with Normasol, pH 7.4 and drug injection. Lead 1 ECG was obtained with intradermal electrodes. Two stainless steel EEG electrodes were anchored 3cm apart into the skull along the temporal ridge, positioned not to penetrate the dura. After stabilization, so that blood pressure was constant and the animal was able to maintain himself without assistance, the left anterior interventricular coronary artery was ligated, just past the major branch, approximately 1cm from its origin. In separate studies on rhesus monkeys this ligation procedure caused an ischemic region extending across the interventricular septum equal to 27% of the left ventricular mass, when measured by the radioactive microsphere technique (10).

In addition to arterial blood pressure, EEG, ECG and heart rate which were recorded on all animals, measurement of myocardial segment tension, intraventricular pressure, dP/dt and end-diastolic pressure as well as arterial blood gases, pH and blood glucose

levels were carried out on selected animals. Since this latter information afforded little data correlative with the recovery or death of the animal from cardiogenic shock, it will not be referred to further in this paper.

Following coronary ligation, ventricular fibrillation (VF) occurred spontaneously in approximately half the animals in 10 to 20 minutes. In those animals that did not fibrillate within the 20 minute period after ligation, VF was induced electrically. VF was permitted to continue for specified time periods ranging from 4 to 24 minutes.

AT the end of the prescribed period resuscitation procedures were started consisting of (a) intra-cardiac injection of 500µg norepinephrine, (b) cardiac massage and (c) electrical defibrillation. The PGB_x treated monkeys received the same resuscitation regimen as the controls but with the additional intra-cardiac injection of 1 mg/kg PGB_x followed by cardiac massage and electrical defibrillation. For these experiments the sodium salt of PGB_x was dissolved in Normasol R to a concentration of 10 mg/ml.

Once the electrical and contractile activity of the heart were reestablished, the animal was allowed to recover spontaneously. If the animal remained in shock, norepinephrine (1-10µg) was infused intravenously until the animal attained a blood pressure level over 40/20mm Hg. or became refractory to norepinephrine and died. If the monkey recovered from the first 4 min. fibrillation and became stable for a period of 20 to 30 minutes, the animal was subjected to the next higher fibrillation period of 6 minutes.

In this manner animals were subjected sequentially to episodes of fibrillation of 4, 6, 8, and 12 minutes duration with 20-30 minute recovery periods until the animal died in shock or successfully survived the course. The last group of animals which recovered from 12 minutes of VF were subjected to 24 minutes of VF.

Paired control and treated monkeys were run on the same day. When a control was run in the morning of one day and a treated animal in the afternoon, the order was reversed with the next pair. In so far as possible, selection of the animals was random except that in four instances, control monkeys, that had failed and could not be brought out of shock with NE, were then given PGB_x and revived to survive the sequential fibrillation series to 24 minutes.

To permit evaluation of the cardiovascular shock or recovery with PGB_x over a period of time after one ischemic event, another series of studies were made with African Green monkeys taken to shock state by a single fibrillation episode of either 8 or 12 minutes. Recovery procedures were the same as with the sequential fibrillation study except that the blood pressure levels in those animals that survived the initial fibrillation period were monitored for at least two hours. In general those animals that failed in shock did so within the first hour after defibrillation. This also permitted the determination of the ability of norepinephrine to maintain blood pressure levels in the presence or absence of

PGB_x and thus evaluate the synergistic effects of PGB_x and norepinephrine.

For the electron microscopic studies, rat liver mitochondria were isolated by differential centrifugation in 0.3M sucrose containing 0.001M versene pH 7.4. The freshly isolated mitochondria were centrifuged at 6000 G, the sucrose removed and the pellet layered with cold 5% glutaraldehyde in 0.1M phosphate buffer pH 7.4. At 30 minute intervals the fixing reagent was changed 3 times, and the mitochondria, packed in ice, were sent for electron microscopy. For the control and PGB_x experiments, 4 mg of fifth day mitochondria were incubated in a mixture containing 0.1 ml of 0.1 M potassium phosphate buffer pH 7.4, 0.16 ml of 0.2 M sodium α-ketoglutarate pH 7.4, 0.1 ml of 0.1 M Mg SO_4 in a total volume of 2.01 ml for 15 minutes in a shaker-bath at 27°C. At the end of the preincubation period, the assay for phosphorylation was begun by the addition of 0.15 ml of a mixture of 0.05 ml 0.1 M ADP, 0.05 ml 0.1 M AMP and 0.05 ml of 2 M KCl, followed immediately by the addition of 0.04 ml of 3.75% solution of crystalline bovine serum albumin to give a final mixture of 2.2 ml. Five control vessels, and five vessels containing 10μg PGB_x in addition, were reacted for 20 minutes with constant shaking. The reaction vessels were pooled in ice cold tubes and centrifuged at 10,000 G for 10 minutes. The supernatants were removed and analyzed for phosphate esterified (11). The pellets then were fixed with cold buffered glutaraldehyde as before.

The fixed pellets from each of the three samples were minced with a fresh degreased razor blade and the resulting segments were immersed in Millonigs-phosphate buffer (MPB) and fixed further with 1.0% OsO_4 in the MPB for one hour. Fixation was followed by rinsing in several changes of MPB and dehydration was accomplished in a graded series of alcohols prior to embedding in Epon. Thin sections were obtained of first day mitochondria, fifth day mitochondria degenerated for 15 minutes and reacted for oxidative phosphorylation for 20 minutes, and fifth day mitochondria plus PGB_x degenerated for 15 minutes and reacted for oxidative phosphorylation for 20 minutes.

For the electron microscopic studies of heart tissue, the aorta was cannulated and the beating heart was perfused retrograde at a pressure of 110 cm H_2O initially with buffered saline and subsequently with 1.25% glutaraldehyde buffered with 0.08 M sodium cacodylate and 0.03 M $CaCl_2$ (pH 7.4). Small tissue samples were obtained from normal, marginal (peri-ischemic) and ischemic regions and were immersed in the same fixative for a period of a ½ hour. Tissues were further trimmed to segments measuring about 0.5 mm^3 and post-fixed in 1.0% OsO_4 (0.1M cacodylate with 3.0% sucrose) at 4°C and brought to room temperature for a total post fixation of one hour duration. The tissue was rinsed 2 times in 0.2 M cacodylate buffer dehydrated in a graded series of alcohol solutions (30, 60, 90, 100 and 100%) followed by two changes in propylene

oxide, impregnated and embedded in epoxy. Thin sections of the embedded tissue were cut with a Sorval MT2B ultramicrotome fitted with a diamond knife and subsequently post-stained with uranyl acetate and lead citrate. For each study, nine electron images of each of six examples were recorded at electron optical magnifications of 3,400 X and 8,200 X employing an RCA EMU-4 electron microscope. All the EM observations and interpretations were made by Dr. John T. Stasny.

RESULTS

A. Studies in Rhesus

1. Initial Fibrillation. Table 1 summarizes the results of the survival of the rhesus monkeys subjected to periods of fibrillation. A total of 10 control and 14 PGB_x treated animals were subjected to an initial episode of VF of 4 minutes duration. At the end of this period, 6 of the controls and all 14 of the PGB_x treated recovered. This difference is statistically significant at $P < 0.02$ (Fisher's exact test).

2. Sequential Fibrillation. After the initial episodes, animals were allowed to recover and were subjected to progressively longer periods of VF of 6, 8, 12 and 24 minutes. As shown in Table 1, the cummulative survival in the controls decreased from 60% at 4 minutes to 25% after 12 minute episodes. In the PGB_x treated group, the 100% survival rate after 4 minutes of VF was maintained at 88% after the sequential exposure to 6, 8 and 12 minutes of VF. This difference is statistically significant ($P < 0.01$ by chi square test).

Six of the PGB_x treated animals that survived the 12 minute period were exposed to 24 minutes of VF. Of these, 5 survived. Of the four controls that survived 12 minutes of VF, two were tested at 24 minutes. One of these survived. Specific statistical comparisons of the survivors after 24 minutes of VF is not possible since not all the animals surviving the 12

Table 1

Survival of Control and PGB_x Treated Monkeys

Subjected to Incremental Periods of Fibrillation

after Left Anterior Coronary Artery Ligation

Control							PGB_x Treated					
	Fibrillation minutes					Time		Fibrillation minutes				
Monkey Number	4	6	8	12	24		Monkey Number	4	6	8	12	24
18C	F						14E		F			
31C	F						15E		S	S	S	
35C	F						19E	S	S		S	
37C	F						20E	S	S	S	S	
32C	S	F					21E	S	S	S	S	
33C	S	S	F				22E	S	S	S	S	
24C	S	S	S	S			23E	S	S	S	S	
45C	S		F				27E	S	S	S	S	
11C		F					29E	S	S	S	S	
12C		F					36E	S	S	S	S	
13C		S	S	S			38E	S	S	S	S	S
16C		S	S	F			39E	S	S	S	S	F
17C		F					40E	S	S	S	S	S
26C		F					41E	S	S	S	S	S
25C	S	S	S	S	F		42E	S		S	S	S
44C	S		S	S	S		43E	S		S	S	S
Cumulative Percent Survival												
	60	44	31	25				100	94	94	88	

(F) indicates monkey failed to recover from the fibrillation and sustain a blood pressure above shock. (S) indicates successful recovery out of shock for the trial period.

minutes of VF were tested at 24 minutes (Table 1).

In addition to the difference in survival, it was noted that most of the treated animals required one electric shock and limited cardiac massage for recovery, while the controls often required repeated shocks and considerable cardiac assistance. Many of the PGB_x treated animals defibrillated spontaneously and required repeated electrical VF to maintain the fibrillation for the experimental period. This was rarely observed in the controls. Table 2 summarizes the frequency of spontaneous defibrillation in control and PGB_x treated monkeys.

B. Studies in African Green Monkeys.

1. Initial VF. Studies involving a single prolonged episode of VF were made in 14 control and 14 PGB_x treated African Green monkeys. Two different groups of animals were exposed to a single episode of either 8 or 12 minutes of VF. The results, summarized in Table 3, were similar to those obtained in the sequential VF studies in rhesus.

In the PGB_x treated group, 89% recovered after 8 minutes of VF and 100% after 12 minutes. This contrasts with 33% and 40% survivals in the controls. The differences are statistically significant ($P < 0.05$). The combined survival (8 and 12 minutes) was 93% for the treated as compared to 36% for the controls ($P < 0.01$).

This experimental design, involving a single episode of VF

Table 2

Spontaneous Recovery from Ventricular Fibrillation After Induced Myocardial Infarction in Rhesus Monkeys

Fibrillation Time Minutes	Incidence of Spontaneous Defibrillation (Percent)	
	Controls	PGB_x Treated
4	0	8
6	7	34
8	7	39
12	0	29
Mean	3.7	28

Table 3

Survival of Control and PGB_x Treated African Green Monkeys After a Single Fibrillation Episode

	Control		PGB_x Treated	
Period of VF	No. Surviving / Total Tested	% Survival	No. Surviving / Total Tested	% Survival
8 minutes	3/9	33	8/9	89*
12 minutes	2/5	40	5/5	100*
Total	5/14	36	13/14	93**

*P < 0.05

**P < 0.01

made possible extended observation on the cardiovascular status of the animal after VF for a period of 2-3 hours. It was noted that a significant number of the control animals, which recovered cardiac activity after initial resuscitation, rapidly deteriorated into a state of circulatory shock. During this period, repeated administrations of NE to these controls resulted in short-lived pressor effects which became progressively less (and/or required higher doses of NE) until no effect could be obtained with even very large doses of NE. In contrast, many PGB_x treated animals responded to NE with a pressor effect which did not return to the previous baseline. Thus in the PGB_x treated group, progressively smaller doses of NE were needed to maintain an adequate blood pressure and eventually the pressure was maintained without any exogenous NE.

2. Infusions of Catecholamines after Resuscitation. To evaluate this phenomenon more directly, a series of experiments were carried out where animals were infused continuously with NE following a period of VF and the infusion rate was adjusted to maintain a diastolic pressure of 60mmHg during the post fibrillation period. The doses of NE required to achieve this ranged from 0.3 to 300µg/min. Six control and 12 PGB_x treated African Green monkeys were studied after the initial resuscitation. In 5 out of 6 controls (83%) the amount of NE infused had to be progressively increased to maintain the desired pressure. In 4 of these animals,

the pressure could not be maintained and the animals died. By contrast, in 7 out of 12 PGB_x treated animals the amount of NE infused necessary to maintain 60mmHg was progressively decreased, and all 12 animals survived.

In many other experiments with control animals that had shock levels of blood pressure, the administration of repeated doses of NE did not produce a pressor effect. When these animals were treated with PGB_x, subsequent administration of NE at the previously ineffective dose levels, usually produced distinct pressor responses.

3. Catecholamines and PGB_x. In initial studies, it was found that intracardiac administration of norepinephrine (NE) during the resuscitation period improved greatly the incidence of recovery and the subsequent status of the animal in both control and PGB_x treated preparations. Therefore, intracardiac NE was included as a standard measure in the studies reported here. Observations made during these initial and subsequent studies suggested that there was a potentiating effect between PGB_x and catecholamines. To determine whether PGB_x potentiated any of the cardiovascular effects of NE in normal animals, 3 normal anesthetized monkeys (Greens) were investigated for dose-response pressor and cardio-accelerator effects of NE (2-5µg/kg) before and after PGB_x. These did not demonstrate any potentiating effect. However, in the animals with blood pressure at shock

levels, pretreatment with PGB_x produced a distinct potentiation of the pressor actions of NE. An example of this effect is shown in Figure 1.

C. Electron Microscopic Studies.

1. In-vitro studies. In an effort to complement the kinetic studies in-vitro, and the recovery studies in-vivo with correlative changes in mitochondrial structure, electron microscopy was carried out on isolated mitochondria subjected to degenerative conditions in the absence and presence of PGB_x. These are compared with the sections of heart from areas considered normal, bordering the infarct area, and from the infarct area from control and PGB_x treated monkeys, after coronary artery ligation and fibrillation for 12 minutes followed by the resuscitation procedures.

The EM observations made in the three mitochondrial preparations are illustrated in Figure 2. Figure 2A represents normal 1st day mitochondria which shows the intact and homogenous condition of the original isolated preparation. This is consistent with the high phosphorylation ability of this preparation (6μmoles of inorganic phosphate esterified per 4 mg mitochondria for the 20 min. reaction period). Figure 2B shows mitochondria degenerated for 15 min. and reacted under conditions for oxidative phosphorylation. This preparation esterified only 0.32μmoles of inorganic phosphate and served as the control. The mitochondria

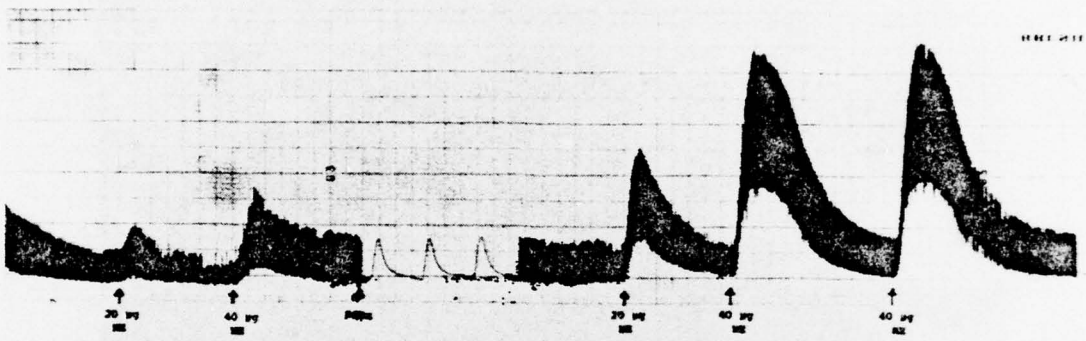

Figure 1. Potentiation of the Pressor Effect of Norepinephrine (NE) following Treatment with PGB_x. This animal had shock levels of pressure and negligible responses to 20 and 40 µg doses of NE. The same doses of NE produced marked pressor responses when tested within 3 to 5 minutes after the administration of 10 mg of PGB_x.

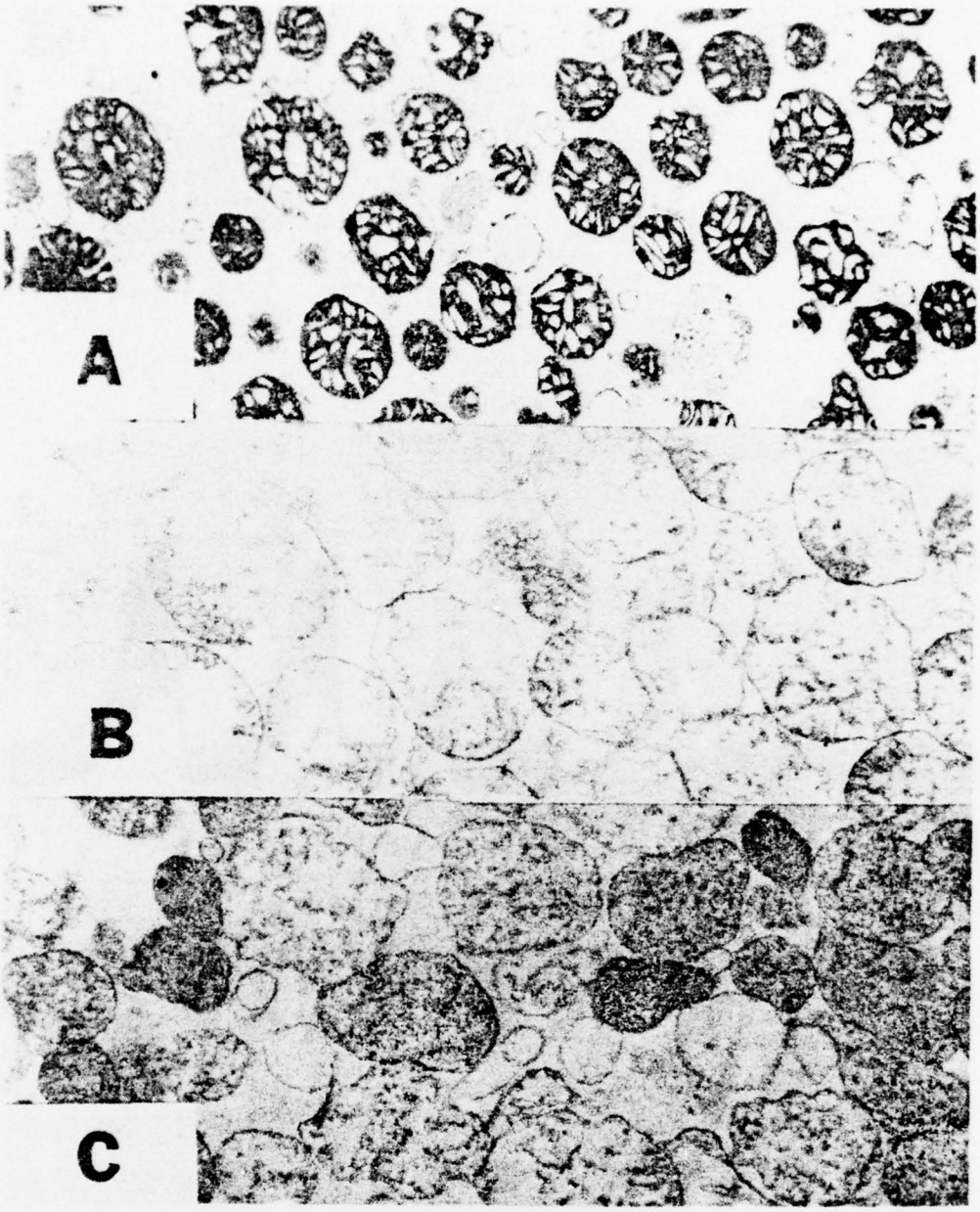

Figure 2. Transmission Electron Microscopy Observations on Isolated Rat Liver Mitochondria. (A) Represents first day mitochondria which have excellent homogeneity. Almost all the mitochondria are in state III or the condensed configuration which represents the low energy state of isolated rat liver mitochondria. The matrix material is very dense. Only very few mitochondria are not in the condensed state. Some microsomes also can be seen. (B) The fifth day mitochondria illustrated here also shows some good homogeneity. However, all these mitochondria are swollen 2 to 3 times the size of the first day mitochondria, but do not appear lysed and show only a very small amount of granular intramitochondrial content and almost no remnants of cristae. (C) These are fifth day mitochondria treated with PGB_X which are less swollen than the untreated mitochondria (B) and contain more matrix material and membranous derivatives of cristae. The small mitochondria appear more dense than the swollen mitochondria. All three plates have a magnification of 20,000X.

in the EM of Figure 2C are similar to the control (2B) except that 10μg of PGB_x were added to the reaction mix. In the presence of PGB_x, oxidative phosphorylation was conserved and the mitochondria esterified 4.36μmoles of inorganic phosphate in the equivalent time. Details of the EM differences are outlined in the legend to Figure 2.

2. In-vivo studies. Tissues for the electron microscopic studies were removed from three untreated and three PGB_x treated animals. Sections were taken from the left ventricle: (a) from a distant, apparently undamaged area in the base of the left ventricle; (b) the border of the infarcted area; and (c) the middle of the infarcted region. Representative sections are shown in Figures 3 and 4. Tissues shown in Figures 3A-3C were taken from an untreated animal after a 12 minute period of VF once it was established that the animal failed to recover. The PGB_x treated animal (Figure 4A-4C) was also exposed to 12 minutes of VF, but survived and was sacrificed after a two hour monitoring period. In general, the mitochondria tissues from PGB_x treated animals are in good condition. Although many mitochondria in the infarcted and border areas are smaller than normal and show bizarre elongated shapes, their matrix density and good condition of membranes suggest structural integrity. This differs considerably from the degenerated, vacuolated condition of the mitochondria from equivalent regions of the untreated animal hearts (Figures 3B and 3C).

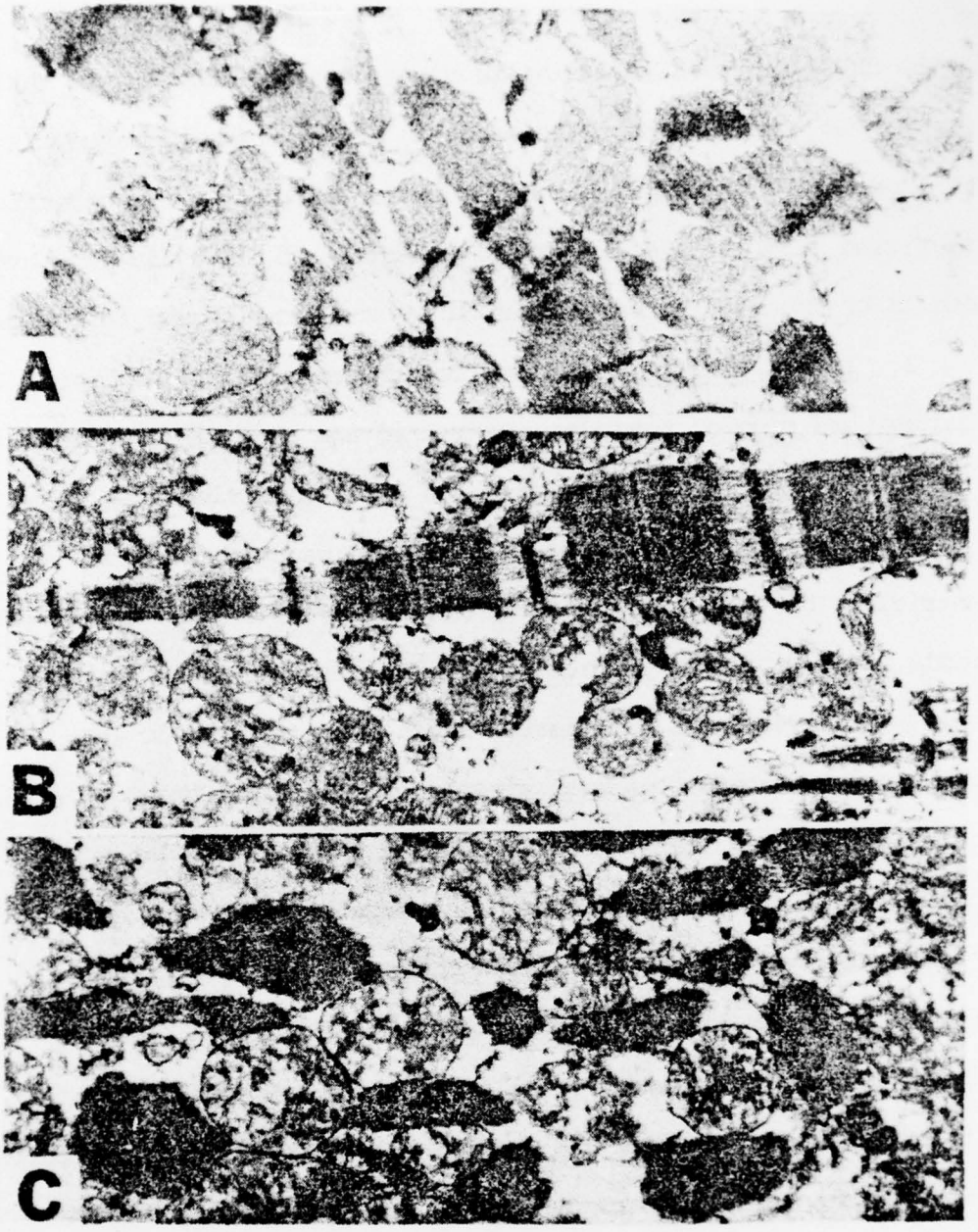

Figure 3. Transmission Electron Microscopy Observations on Tissue Sections from an Untreated Monkey with Myocardial Infarction after 12 Minutes of VF. (A) Represents the Non-infarcted Tissue from the Base of the Left Ventricle; (B) the Border Zone; (C) the Center of Infarcted Area. (A) In the non-infarcted region, the mitochondria in some areas are well preserved while in others they are lacking in matrix density but show intact cristae which are prominent and numerous. (B) In the border zone, the mitochondria are broken and leached of matrix material. (C) In the infarcted area, the mitochondria are swollen, highly disorganized, and deteriorated. The cristae are in short segments within a leached matrix. Magnification in all three sections is 24,600X.

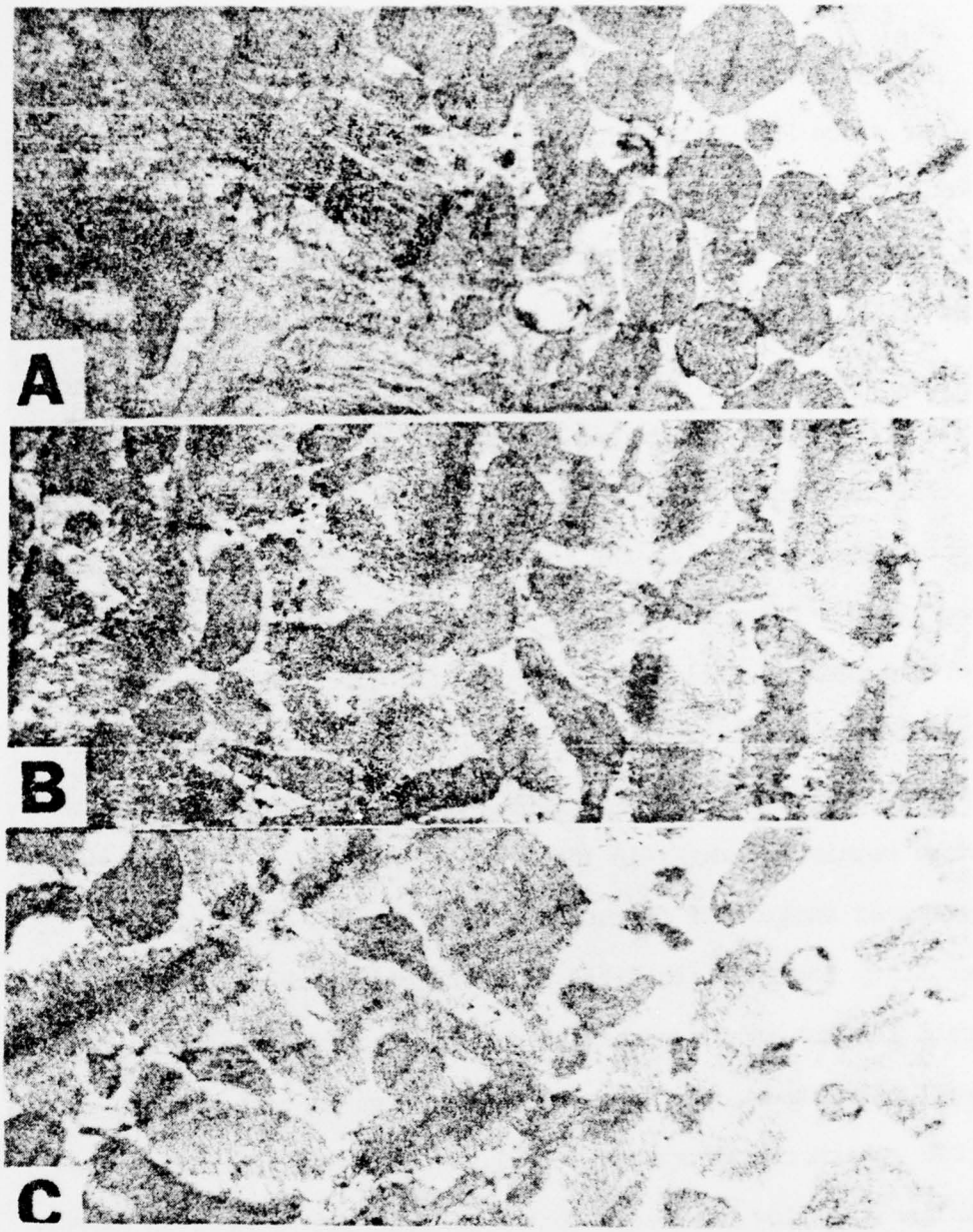

Figure 4. Transmission Electron Microscopy Observations on Tissue Sections from a Monkey which was Treated with PGB_x after 12 Minutes of VF. (A) Represents the Non-infarcted Tissue from the Base of the Left Ventricle; (B) the Border Area; (C) the Center of the Infarcted Area. (A) In the non-infarcted region, the mitochondria matrix is, in general, dense and in good condition. (B) In the border zone, the mitochondria are numerous, intact and dense but are pleomorphic. (C) In the infarcted tissue, the mitochondria are similar in condition to those in the border zone (B); they are numerous and small and some show unusual shapes but are nevertheless intact and dense. Magnification in all three sections is 24,600X.

Discussion

The primary hypothesis underlying the experiments described is that since PGB_x has a unique in-vitro action in the conservation of oxidative phosphorylation under conditions degenerative to mitochondria, a similar action in-vivo should serve to enhance the survival of an ischemic anoxic crisis which incurs mitochondrial damage. This implies that cardiac muscle contraction is limited by the rate at which chemical energy can be supplied by the metabolic process. Although there is ample evidence (1) for biochemical and morphological changes in the infarcted myocardium with considerable damage in the forms of vacuolation, swelling and loss of crystalline structure seen in mitochondria, it is important to remember that a distinction exists between biochemical and structural damage. Mitochondria,which may take a long time to recover morphologically, seem to regain and maintain their functional integrity even though they appear ragged and disrupted (12). Despite these elements of uncertainty for specific sites of dysfunction, there remains the overall failure in the mechanism coupling energy transformation with energy utilization. It is in this mechanism that we propose a role for PGB_x which reacts synergistically with norepinephrine to re-establish the flow of energy to the contractile process in cardiogenic shock. PGB_x can be definitively associated with conservation and reactivation of mitochondrial synthesis of ATP from the in-vitro findings (7). According to Ellis (13) the evidence for norepinephrine action on contraction seems to be at some site

coupling metabolic energy to the contractile process. Catecholamines simultaneously increase ATP breakdown and contractile force (14). Adrenergic mediators also increase the maximum velocity of shortening (15,16). The combined action of PGB_x and norepinephrine then could reestablish both sufficient energy and sufficient utilization to account for the survival after the ischemic crisis and cardiogenic shock.

The repeatedly confirmed findings that PGB_x has no effect or even a small inhibitory action on intact mitochondria in-vitro and no demonstrable effect on the normal animal cannot be overemphasized. The dramatic actions of PGB_x occur only with damaged mitochondria in-vitro or after ischemic pathology in-vivo. The effects observed suggest that PGB_x can replace or bridge an essential factor in energy transformation lost when mitochondria are swollen and damaged. In this respect it resembles a vitamin functioning as coenzyme which has little effect on the coenzyme saturated system but markedly activates the coenzyme depleted system.

The results of the studies obtained in both Rhesus and African Green monkeys indicate that treatment with PGB_x greatly improved the incidence of survival after periods of complete circulatory arrest produced by VF. Recovery after circulatory arrest depends initially on the restoration of electrical and mechanical activity of the heart and subsequently on the reestablishment of the cardiovascular control mechanisms responsible for the maintenance of

effective blood pressure levels. Improved recovery is therefore likely to involve primarily cardiac effects followed by reversal of the shock state.

The experimental methods used in the present studies included prior insult to the heart by coronary ligation involving a significant proportion of the left ventricle. Under these conditions improved cardiac resuscitation could be the result of improvements of (a) the general status of the entire heart, or (b) primarily the marginal ischemic regions.

It is well known that the cardiac resuscitation is more difficult in the presence of coronary occlusion (8,9). Therefore, one possible interpretation of the results is that treatment with PGB_x in some way alters the degree of myocardial injury associated with coronary occlusion. This could be the case if PGB_x reduced the size of the metabolically injured myocardium following coronary occlusion. It is now generally believed that the tissue injury produced by coronary occlusion includes a significant portion of marginal tissue with diminished blood flow, the ultimate fate of which depends upon the discrepancy between the tissue metabolic demands and the reduced circulation(17,18,19). The effect of PGB_x in restoring phosphorylating activity of previously damaged mitochondria in-vitro, is consistent with the possibility that the results of the present study could be due to PGB_x actions on the marginal areas of coronary occlusion. However, considering that VF

produces generalized hypoxia in the entire heart, an equally likely action of PGB_x (again based on its action on mitochondria) is that it affects the ability of the entire heart to recover. Most likely, both factors play a significant role in determining the difference in the survival between control and PGB_x treated animals in the present experiments.

Alternative interpretations of the results also should be considered. It is possible that the observed effect of PGB_x in the intact animal is unrelated to its actions on isolated mitochondria and represents unrecognized effects on the sarcoplasmic reticulum or other cellular membranes such as lysosomes. Preliminary in-vitro studies suggest a stabilizing effect on lysosomes isolated with mitochondria. Moreover the in-vitro effect of PGB_x in favoring phosphorylation to Ca^{++} uptake may have its counterpart in regulating excess Ca^{++} in the contractile process of the anoxic myocardium. It is also possible that the observed in-vivo effect may depend on interaction between PGB_x and other naturally occurring compounds. In this connection the potential interaction between PGB_x and circulating catecholamines is of particular interest in view of the observations made during the present studies on the biological interactions between PGB_x and norepinephrine. All these alternates represent plausible speculations which are subordinate to the firm experimental facts of

PGB_x action on mitochondria.

Although PGB_x is a polymeric derivative of prostaglandin B, it has none of the reported activities of any of the known prostaglandins. Both its molecular size and structure which includes a stable free radical component favor a unique metabolic action not shown by the monomeric prostaglandins. It is also unrealistic to expect that PGB_x would be converted metabolically to a monomeric prostaglandin. Therefore, it would be unlikely to have biological properties attributed to the known prostaglandins. PGB_x has no structural similarity to PGX (or PGI_2) recently reported (20,21).

In general the present results favor the view that PGB_x has an activity in-vivo similar to that previously demonstrated in isolated mitochondria. PGB_x then would constitute the prototype of an entirely new class of compounds whose biological activity would involve restoration of metabolic functions following hypoxic or ischemic injury. Pharmacological compounds possessing such an activity would have a broad application in a variety of disease and traumatic states.

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